Enzymic conversion of malto-oligosaccharides and maltodextrin into cyclodextrin at low temperature

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In the presence of appropriate complexants at 5–25 °C, maltose, maltotriose and maltohexaose were converted by *Bacillus macerans* cyclodextrin glucanotransferase (EC 2.4.1.19) into β-cyclodextrin (β-CD, cyclomaltoheptaose) in yields as high as 34, 49 and 66% respectively. In the absence of a complexant, yields of CD were extremely low (<2% overall); however, yields were greatly enhanced by the presence of complexants (cyclononanone, cyclodecanone and cycloundecanone) known to be highly selective for β-CD. Many of the complexants that enhance CD production from starch or maltodextrin failed to enhance CD production from maltose. An investigation of the influence of molecular size and geometry of alkyl alcohols and aliphatic hydrocarbons on CD production from maltodextrin at low temperature revealed that all C_2–C_{14} alcohols and all C_3–C_{15} hydrocarbons enhance overall CD yield. While all of the hydrocarbons were highly selective for β-CD, the selectivity of the alcohols varied according to chain length and degree of branching, with no alcohol enhancing the yield of γ-CD. With the exception of propan-1-ol, straight-chain alcohols with eight or fewer carbon atoms strongly favoured β-CD production; those with more than eight carbon atoms favoured α-CD production. Highest yields of α-CD (49–53%) were obtained with C_9–C_{12} alkan-1-ols. With the exception of n-butane, the use of which provided β-CD yields as high as 59%, use of branched hydrocarbons and branched alcohols resulted in β-CD yields (58–64%) higher than those obtained with the corresponding unbranched compounds.

Introduction

Through the action of cyclodextrin glucanotransferase (CGTase[1]), starches and maltodextrin are converted into a mixture of cyclodextrins [cyclomalto-oligosaccharides; cyclic (1→4)-α-D-glucans; CDs] and non-cyclic malto-oligosaccharides. In the absence of compounds capable of forming inclusion compounds with CDs, the non-cyclic products are normally favoured; overall yields of CD are usually 35–50%. Of the three CDs formed in these reactions [cyclomaltohexaose (α-CD), cyclomaltoheptaose (β-CD) and cyclomalto-octaose (γ-CD)], γ-CD is favoured least, except in those instances where a γ-specific CGTase [1,2] is employed. The relative proportions of α-CD and β-CD can vary appreciably according to reaction conditions.

Product ratios and total CD yields can be greatly influenced by the formation of either highly insoluble or highly stable inclusion compounds in the reaction mixtures. Cyclodextrin inclusion compounds are the complexes formed by the entrapment of a guest molecule (complexant) within the cavity of a CD molecule (the host). Yield enhancement can occur through the influence of aliphatic alcohols [3–8], polyols [9], C_2–C_4 aliphatic ethers, esters and ketones [3], aliphatic hydrocarbons [7,10,11], long-chain aliphatic acids, esters, nitriles, thiols, ethers and halides [7], aromatic compounds [7,12–16], cyclic ketones [10,17–19], or tetra- and penta-cyclic terpenoids [20]. In most, if not all, instances of yield enhancement, the increases in yield result from complexation. Only with certain water-soluble, low-molecular-mass compounds (such as ethanol and acetone) [5] might there be uncertainty regarding the contribution of complexation to yield enhancement. Except for several investigations conducted at 30 °C [21–23], reaction temperatures chosen for the production of CDs have been almost exclusively in the range 40–60 °C. Little, if any, attention has been devoted to temperatures in the 0–25 °C range. Consequently, a need existed for knowledge concerning the influence of low temperature on conversion yields in the absence or presence of complexant.

[1] Abbreviations used: CD, cyclodextrin; CGTase, cyclodextrin glucanotransferase; d.e., dextrose equivalent; dp, degree of polymerization; $\bar{d}p$, average degree of polymerization; G_1, D-glucose; G_2–G_{10}, straight-chain malto-oligosaccharides of dp 2–10.

The present paper describes how temperatures in the range 5–25°C affect overall yields and proportions of α-, β- and γ-CDs produced in the reaction of *Bacillus macerans* CGTase with maltodextrin and selected malto-oligosaccharides at pH 7 in the absence or presence of complexing agents. Studies of the influence of molecular size and configuration of aliphatic hydrocarbons and aliphatic alcohols as complexing agents on CD yields are described, and the results are compared with those obtained by other investigators using similar guest compounds at higher temperatures. Such information might serve to reduce the cost of producing CDs industrially. Lower costs would promote greater interest in the unique ability of these cyclic compounds to encapsulate and thereby modify the physical and chemical properties of a great variety of guest molecules. The complex-forming properties of CDs are of interest both for basic research as well as for practical applications in food, pharmaceutical, cosmetic and agricultural industries.

Experimental

Materials

CGTase from *Bacillus macerans* was obtained as an aqueous solution (>600 units/ml, according to the method of Tilden and Hudson [24]) from Amano International Enzyme Co., Inc., Troy, VA, U.S.A. In 30 min at 60 °C and pH 6.0 (adjusted by means of NaOH and HCl), a single 5 μl application of the CGTase (equivalent to 3 units of activity) to a maltodextrin mixture of average degree of polymerization (d̄p) 22.1 (0.3 g of maltodextrin in 3 ml of water in a capped glass culture tube) produced 16.5 mg of combined CDs (yields: 2.9% α, 1.8% β and 0.8% γ). Amyloglucosidase (1,4-α-D-glucan glucohydrolase, EC 3.2.1.3; from *Aspergillus niger*; 37 units/mg of solid or 42 units/mg of protein; 1 unit liberates 1.0 mg of D-glucose from starch in 3 min at pH 4.5 and 55 °C) was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.. The maltodextrin M-040 [7.7% water; dextrose equivalents (d.e.) 5; d̄p 22.1] and corn-syrup solids M-250 (5.4% water; d.e. 25; d̄p 4.5) and M-255 (6.1% water; d.e. 25; d̄p 4.5) were from Grain Processing Corporation, Muscatine, IA, U.S.A. β-CD (13.7% water) and corn-syrup solids Fro-Dex 24 (5.96% water) and Fro-Dex 42 (5.8% water) were from American Maize Products Co., Hammond, IN, U.S.A. α-CD (8.8% water) and γ-CD (9.0% water) were from Anspec Co., Inc., Ann Arbor, MI, U.S.A. Oligosaccharide-free maltose was prepared from maltose octa-acetate [25]; other malto-oligosaccharides (the purest available) were from Aldrich Chemical Co., Milwaukee, WI, U.S.A. The purity of alkyl alcohols (Aldrich Chemical Co.) was at least 99%, except in the case of 4-methylpentan-1-ol, octan-2-ol, tetradecan-1-ol, and tridecan-1-ol, where purity was 97–98%. Purity was greater than 99% for all hydrocarbons (Aldrich Chemical Co.), except propane (98%), n-butane (98%), 2-methylpropane (99%), cyclopentane (95%) and cyclohexane (98%). Cyclic ketones (Aldrich Chemical Co.) were the purest available (96–99%). Water was distilled and deionized. Values for d.e. and d̄p of M-040 and corn-syrup solids were provided by the manufacturers.

The manufacturer of M-040, M-250 and M-255 provided information on the average saccharide composition of these three substances. For anhydrous M-040, in % by weight (where G_1 is D-glucose and G_2–G_{10} are straight-chain malto-oligosaccharides of dp 2–10): G_1 0.2, G_2 0.3, G_3 0.6, G_4 0.6, G_5 0.6, G_6 1.0, G_7 1.2, G_8 0.8, G_9 0.5, G_{10} 0.3, and dextrins of dp >10, 93.9. For anhydrous M-250: G_1 8.3, G_2 7.3, G_3 7.0, G_4 6.3, G_5 5.4, G_6 4.3, G_7 3.3, G_8 2.9, G_9 2.1, G_{10} 2.2, and dextrins of dp >10, 50.9. For anhydrous M-255: G_1 2.4, G_2 8.1, G_3 9.5, G_4 6.0, G_5 5.7, G_6 13.7, G_7 9.6, G_8 1.2, G_9 0.8, G_{10} 0.7, and dextrins of dp >10, 42.3. The D-glucose contents of Fro-Dex 24 and 42 were 13.8% and 25.1% respectively; the respective maltose contents were 8.3% and 14.7% [19].

The CGTase used in these studies was described by the manufacturer as being most stable above pH 7, with stability decreasing rapidly below pH 6. Activity decreases rapidly as pH increases above 7 and as pH decreases below 5. At pH 7, the thermostability of the enzyme is extremely high at or below 30 °C (no detectable change in activity over a period of 10 min), moderately high at 50 °C (~99% of the activity remaining after a 10 min treatment), but relatively low at 60 °C (~60% of activity remaining after 10 min; or about 3% after 8 h).

Analytical methods

CDs and low-molecular-mass saccharides (G_1–G_7) were separated by HPLC on a DuPont Zorbax NH_2 column (4.6 mm × 250 mm) at 40 °C with acetonitrile/water (13:7, v/v) at 1.0 ml/min and detected by refractometry. The CD reference standard for comparison with elution peaks was a mixture of α-, β-, and γ-CDs (0.80 mg of each anhydrous CD/ml of aqueous solution); the saccharide reference standard contained G_1–G_7, with each compound at 1 mg/ml. All solutions were filtered by syringe through Millipore HV filter units of 0.45 μm pore size (Millipore Corp., Bedford, MA, U.S.A.) prior to injection. Prior to HPLC analysis for CDs, reaction mixtures containing malto-oligosaccharides were treated with amyloglucosidase at pH 4.5 and 55 °C to eliminate malto-oligosaccharides that would interfere with CD determinations. Calculation of CD yields was based on the total amount (mmol) of glucose residues in the substrate.

Conversion procedure

Except for reactions involving liquified gases, 2 ml portions of substrate solution [usually 10% (w/v) carbohydrate in water] were placed in individual screw-capped culture tubes (with or without complexant, as required), the pH was adjusted to 7.2 (with HCl or NaOH) by means of an Aldrich thin-stem (3.5 mm) pH electrode (Aldrich Chemical Co.), and an appropriate amount of CGTase was added to each. Reactions at 50 and 60 °C were conducted in constant-temperature shaker baths. At lower temperatures, magnetic stirring in constant-temperature rooms was employed. Where possible, periodic adjustment of pH was made. Procedural requirements often necessitated the application of CGTase in several small portions (increments), the frequency of addition being dependent upon reaction temperature. Enzyme inactivation was conducted at 100 °C

Where liquifiable gases were employed, the procedure for preparing reaction mixtures differed significantly from that given above. A 4 ml portion of 10% (w/v) substrate solution, an appropriate amount of CGTase and a short magnetic stir bar were placed together in a glass high-pressure reaction tube (Ace Glass Inc., Vineland, NJ, U.S.A.) equipped with a Teflon screw plug. The lower end of the tube was then immersed in a liquid-nitrogen bath in order to freeze the contents and permit the liquification of gaseous n-propane, n-butane or 2-methylpropane (introduced by means of a length of Tygon tubing) on to the surface of the frozen solution. With the Teflon plug in place, the tube was subsequently allowed to warm up to the desired reaction temperature (the temperature was never allowed to rise above the pressure limit of the reaction vessel). Agitation was performed by magnetic stirring. At the conclusion of the reaction, the reaction mixture was frozen with liquid nitrogen before the vessel was opened.

Removal of complexant from reaction mixtures by azeotropic distillation

Mixtures were heated in a water bath to ~95 °C while nitrogen was introduced at a controlled, moderate rate beneath the liquid surface by means of a capillary tube; the duration of the operation varied according to the stability of the complex. The complexant-free solutions were then diluted appropriately and filtered by syringe through Millipore HV 0.45 μm filters prior to HPLC analysis.

Some complexants, because of exceptionally low volatility, are very difficult to remove solely by the technique described above. In such cases, removal was greatly facilitated by extracting the reaction mixture with several portions of diethyl ether prior to distillation. Where liquified gases were employed as complexants, mere warming was sufficient for their removal.

Results and discussion

At 0–25 °C, *Bacillus macerans* CGTase was extremely stable and was found to retain much, if not most, of its original activity during the course of low-temperature reactions described here. This stability was well illustrated by studies of maltose conversion to be described later. Except in those few experiments where a single large-size application of CGTase was made, CGTase was applied in several small portions, generally separated by brief time intervals of 5–7 days in order to facilitate determining when maximum yield was attained. Instead of starch as a substrate for conversion, maltodextrin M-040 was used in order to eliminate problems associated with starch liquification. Maltodextrin M-040 dissolves easily in water, has a very low D-glucose and maltose content, and has a conversion behaviour very similar to that of partially hydrolysed (liquified) starch.

Influence of aliphatic alcohols on conversion of M-040

Some investigators have attributed the enhancing influence of low-molecular-mass alcohols to changes in water activity [8] or to some change in either the substrate or the enzyme that results in inhibition of certain transglycosylation processes [4]. However, the trend in the behaviour of an alcohol as the molecular mass and structural complexity are increased (see Table 1) suggests that, at 25 °C, alcohols larger than methanol act as guest molecules and that their complexation with CDs is the primary cause of variation in CD yields. At 5 °C, even methanol possesses significant ability to enhance yields. Consequently, in this discussion, the influence of alcohols will be viewed as being caused primarily by the formation of inclusion compounds, although one must bear in mind that changes in water activity, changes in degree of hydration of the substrate and enzyme, and changes in the infra-structure of the substrate and enzyme, all caused by the presence of alcohol, might be contributing factors.

In a homologous series of alkan-1-ols (see Table 1), beginning with methanol, the combined yield of CDs at 25 °C increased with increasing molecular mass until it reached a relatively constant value at butan-1-ol; however, a small but definite decrease in yield occurred with alcohols larger than undecan-1-ol. Although data for reactions at 5 °C are less extensive than those at 25 °C, it is apparent that the relationship between combined yield and molecular mass at 5 °C is very similar to that at 25 °C. Also, in none of the reactions was γ-CD produced in larger than trace amounts. Interestingly, only C_9–C_{12} alkan-1-ols gave high ratios of α-CD to β-CD. Similar high ratios for these four alcohols were found earlier by Armbruster and

Jacaway [7], who investigated the influence of alcohols on starch conversion at 50 °C. With decan-1-ol at 50 °C, yields of α- and β-CDs were 45% and ≈2% respectively. Other workers [26] have also reported α-CD yields close to 50%. In the present work, yields of α-CD at 25 °C were 52 and 55% respectively. No adequate explanation can be offered at this time for the pronounced ability of C_9–C_{12} alkanols to enhance α-CD production. That a hydroxy substituent on the terminal carbon atom of a long-chain aliphatic hydrocarbon plays an important role in this high selectivity is indicated by a tendency for certain polar substituents other than a hydroxy group to favour α-CD formation [7]. Among these substituents are aldehyde [7], carboxy [7] and sulphate [27] groups. Replacement of these substituents with hydrogen eliminates specificity for α-CD and promotes β-CD production.

A tertiary alkyl group (as in 2-methylpropan-2-ol) or an isopropyl substituent at the terminal end of an alkanol (as in 2-methylpropan-1-ol, 3-methylbutan-1-ol, 4-methylpentan-1-ol, 6-methylheptan-2-ol and 3,7-dimethyloctan-1-ol) promotes greatly the ability of an alkanol to enhance β-CD production (Table 1). This high specificity is possibly caused by a larger amount of contact that branched alkyl moieties make with the walls of a β-CD cavity relative to the amount of contact that straight-chain moieties would make.

It is noteworthy that no precipitation occurred at either 5 or 25 °C in reaction mixtures containing alcohols having fewer than five carbon atoms. With hexan-1-ol, a precipitate formed at 5 °C, but not at 25 °C. Precipitates were observed in all reactions containing alcohols larger than hexanol. It is very probable that these precipitates were CD–alkanol complexes and that the absence of precipitation in reaction mixtures containing C_1–C_6 alkanols resulted not from an absence of complexation but from the high solubility of a highly stable complex. Combined CD yields at 25 °C from reactions conducted in the presence of C_4 alkanols are very similar to yields obtained

Table 1 Influence of molecular size and geometry of aliphatic alcohols on conversion of M-040[a] to CDs at low temperature

	CD yield (%)							
	25 °C[c]				5 °C[d]			
Alcohol[b]	α	β	γ	Combined	α	β	γ	Combined
None[e]	8.3	16.0	2.7	27.0	4.6	7.9	2.1	14.6
Methanol	14.2	15.9	2.3	32.4	10.4	12.2	1.7	24.3
Ethanol	24.9	16.6	1.3	42.8	24.9	15.8	2.0	42.7
Propan-1-ol	31.6	21.9	0.2	53.7	29.2	25.8	1.0	56.0
Propan-2-ol	12.9	37.5	0.4	50.8				
2-Methylpropan-2-ol (t-butyl alcohol)	2.6	57.2	0	59.8				
2-Methylpropan-1-ol	9.4	46.8	0.2	56.4				
Butan-1-ol	30.7	29.0	0	59.7	29.6	31.4	2.0	63.0
Butan-2-ol	18.4	39.2	0.2	57.8				
Pentan-1-ol	33.2	26.8	1.0	61.0	36.7	17.9	0.4	55.0
3-Methylbutan-1-ol	8.3	51.9	0.2	60.4				
Hexan-1-ol	36.2	22.9	0	59.1	11.0	55.0	1.8	67.8
Hexan-2-ol	33.2	24.1	0.2	57.5				
4-Methylpentan-1-ol	7.5	5.2	0.5	60.2				
Heptan-1-ol	21.8	36.7	0.2	58.7	5.7	58.8	2.5	67.0
Octan-1-ol	13.2	42.1	0	56.3	26.5	37.6	1.9	66.0
Octan-2-ol	1.6	45.2	0	57.8				
6-Methylheptan-2-ol	1.0	59.5	0	60.5				
Nonan-1-ol	49.6	6.6	0	56.2				
Decan-1-ol[e]	51.9	3.8	0	55.7	54.9	3.3	0	58.2
3,7-Dimethyloctan-1-ol	0.5	62.0	0	62.5				
Undecan-1-ol	52.8	2.7	0	55.5				
Dodecan-1-ol	49.0	2.7	0	51.7				
Tridecan-1-ol	33.1	11.8	0.2	45.1				
Tetradecan-1-ol	36.5	11.3	0.2	48.0				

[a] Volume of 10% (w/v) M-040 solution, 2.00 ml; 1.234 mmol of glucose residues.

[b] 13% (v/v) of reaction mixture, except with the solid alcohols (dodecan-1-ol, tridecan-1-ol, tetradecan-1-ol), where 0.2 g of alcohol was present in each 2 ml of reaction mixture.

[c] Unless noted otherwise, the total reaction period was 10 days, and two 7 μl increments of CGTase (each is equivalent to 4 units of activity) were applied at a frequency of one increment per 5-day period; no more than two increments were required for attainment of reaction 'equilibrium'.

[d] The reaction period was 20 days; single 21 μl application of CGTase (equivalent to 13 units of activity) for mixtures containing alcohol; single 15 μl application of CGTase (equivalent to 9 units of activity) for mixture without alcohol.

[e] At 25 °C, a reaction time of 5 days and a single 7 μl application of CGTase (equivalent to 4 units of activity) were used; more than one application resulted in lower yields of α-CD.

with higher alcohols [7] known to form insoluble CD complexes, suggesting that yield enhancement with C_4 alkanols might also involve the formation of a complex.

Influence of hydrocarbons on conversion of M-040

Table 2 presents results of an investigation of the influence of hydrocarbons at temperatures ranging from 5 to 25 °C. In several studies, for reason of comparison, higher temperatures of 50 and 60 °C were employed. The maximum ring size of cyclic hydrocarbons was limited to eight members. In all conversion experiments with either aliphatic or cyclic hydrocarbons, β-CD production was highly favoured. Also, little difference was noted between combined yields or proportions of α-, β- and γ-CDs obtained at 5 °C and those obtained at 25 °C. That the use of the much higher temperature of 50 °C can, in some instances, lead to smaller yields is exemplified by the behaviour of n-butane and, to a lesser degree, cyclopentane. At 25 °C, the ring size of cyclic hydrocarbons had little influence on maximum yields. Among the unbranched straight-chain alkanes at 25 °C, n-butane provided the highest combined CD yield (58.6%); yields from the use of propane and C_5–C_{15} n-alkanes lay within the range 41–53%. The high overall yields (59–64%) from the use of branched alkanes (2-methylpropane, 2-methylbutane and 2,2,4-trimethylpentane) probably resulted from their being able to fit within the cavity of β-CD more snugly than could most straight-chain alkanes. It is a curious fact that unbranched n-alkanes, especially those of low molecular mass such as propane and n-butane, do not favour α-CD production. Two possible explanations for this behaviour can be offered: (1) because of the influence of the surrounding aqueous (polar) environment, intramolecular attraction resulting from hydrophobic forces causes hydrocarbon chains to curl up into ball-like masses, the size and shape of which permit preferred accommodation of guests within the β-CD cavity, and (2) because of the aqueous environment, two or more hydrocarbon chains associate through intermolecular hydrophobic attraction to form a unit of a size and shape that is easily and preferentially accommodated by a β-CD cavity.

At 50 °C, the use of aliphatic and alicyclic hydrocarbons having six, eight, ten and twelve carbon atoms enabled Armbruster and Jacaway [7] to produce β-CD either exclusively or almost exclusively. Although their yields at 50 °C with cyclic hydrocarbons were very similar to yields obtained in this laboratory at 25 °C, yields at 50 °C with n-octane (~30%) and n-decane (~25%) were considerably smaller than the corresponding yields at 25 °C. The enhancing effect that lower temperatures can have on CD yields in systems containing low-molecular-mass aliphatic hydrocarbons is well illustrated by the behaviour of n-butane at 5, 25, and 50 °C (Table 2).

Conversion of maltodextrin and corn syrup solids in the presence of cyclic ketones

Table 3 presents data on the conversion of maltodextrin M-040 and various corn-syrup solids that differ considerably in d.e., $\bar{d}p$, D-glucose content and maltose content. In the absence of complexing agents, yields of CD at 25 °C from corn-syrup solids were very low compared with the yield from maltodextrin M-040. Yields were enhanced considerably by the presence of complexants, although CD production from corn-syrup solids was still appreciably lower than that from M-040. The extent of production appeared to be strongly influenced by the D-glucose content of the substrate. The higher the D-glucose content, the lower the yield. Maltose probably contributed to low yields, but only where complexants were absent [10,19,19a (the preceding paper)]. The nature of certain high-dp components of the substrates might also have been a yield-determining factor [19]. Lowering of the reaction temperature from 25 to 5°C had little effect, if any, on the extent of conversion in the presence of complexants. However, in the conversion of corn-syrup solids in the presence of β-CD-specific complexants, the use of low temperature is definitely advantageous. At 60 °C, in the presence of cyclodecanone, the maximum conversion of M-250 into β-CD was only 33%, whereas at 25 °C, with the same complexant, the maximum yield was ~46%. On the other hand, in the conversion of maltodextrin into β-CD in the presence of β-CD-specific complexants, yields at low temperature were not significantly better than those at 60 °C [10].

Attempts to obtain more than relatively moderate yields of γ-CD at 25 °C have not been successful. Complexants that are specific for γ-CD production (cyclotridecanone and 8-cyclohexadecen-1-one) were less effective at 25 °C than at higher, more commonly used, temperatures. Yields of γ-CD as high as 50% have been reported for the conversion of maltodextrin (or liquified starch) in the presence of cyclotridecanone at 60 °C [10], and as high as 45% in the presence of 8-cyclohexadecen-1-one at 50 °C [18]. However, at 25 °C the respective yields with these complexants were only 28 and 37% (Table 3).

Conversion of malto-oligosaccharides

Table 4 presents data on the action of CGTase on maltose, maltotriose and maltohexaose at temperatures ranging from 5 to 60 °C. Although HPLC analyses showed that maltose, in the absence of complexant, undergoes exten-

sive interaction with CGTase to produce, through disproportionation, a large array of higher malto-oligosaccharides (maltotriose to maltoheptaose), CD became detectable at 60 °C only after an extremely lengthy reac-

Table 2 Influence of molecular size and geometry of hydrocarbons on conversion of M-040[a] to CDs

Abbreviation: NOI, number of increments.

Hydrocarbon[b]	Temp. (°C)	Time (days)	CGTase NOI	CGTase Increment vol. (μl)	CD yield (%) α	β	γ	Combined
None	5	21	1	15	4.6	7.9	2.1	14.6
	25	5	1	7	8.3	16.0	2.7	27.0
	60[c]	3	3[f]	4	11.0	17.8	4.0	32.8
Non-cyclic								
Propane[d]	10	9	1	28	1.3	46.1	0	47.4
n-Butane[d]	5	21	1	30	1.1	59.2	0	60.3
	25	10	1	14	1.8	56.8	0	58.6
	50	4	1	14	8.3	20.1	2.3	30.7
2-Methylpropane[d]	15	6	1	21	1.0	59.3	0	60.3
n-Pentane	5	21	1	21	1.7	51.6	0	53.3
	25	10	2[e]	7	3.6	43.9	0.5	48.0
2-Methylbutane	5	21	1	21	0.9	58.1	0	59.0
	25	5	1	7	2.1	56.3	0.2	58.6
	25	10	2[e]	7	1.9	54.9	0.2	57.0
n-Hexane	5	21	1	21	1.7	49.1	0	50.8
	25	5	1	7	3.3	43.3	0.5	47.1
	25	10	2[e]	7	3.6	43.9	0.5	48.0
	25	15	3[e]	7	4.3	42.8	0.6	47.7
n-Heptane	25	5	1	7	3.4	48.0	0.8	52.2
	25	10	2[e]	7	2.8	45.7	0.5	49.0
n-Octane	25	10	2[e]	7	2.4	48.2	0.5	51.1
2,2,4-Trimethylpentane (iso-octane)	5	21	1	21	0.6	62.3	0	62.9
	25	3	1	7	8.5	62.6	0	63.1
	25	10	2[e]	7	0.8	63.6	0	64.4
n-Nonane	25	10	2[e]	7	2.1	49.9	0.5	52.5
n-Decane	25	10	2[e]	7	3.6	40.6	0.5	44.7
n-Undecane	25	10	2[e]	7	18.8	22.4	0.4	41.6
n-Dodecane	25	10	2[e]	7	17.3	28.8	1.1	47.2
n-Tridecane	25	10	2[e]	7	2.5	43.2	0.2	45.9
n-Tetradecane	25	10	2[e]	7	2.7	43.6	0.4	46.7
n-Pentadecane	25	10	2[e]	7	2.6	37.8	0.4	40.8
Cyclic								
Cyclopentane	25	10	2[e]	7	0.8	64.0	0	64.8
	50	3	3[f]	3.5	1.9	52.8	0.2	54.9
		6	6[f]	3.5	1.6	52.5	0.1	54.2
Cyclohexane	5	7	1	15	5.6	57.9	0.2	63.7
		21	1	15	2.5	63.1	0.1	65.8
	25	5	1	7	0.9	61.6	0	62.5
		10	2[e]	7	0.6	61.6	0.3	62.2
		20	4[e]	7	1.1	55.8	0	56.9
	50	3	3[f]	3.5	1.3	55.9	0.2	57.4
		6	6[f]	3.5	0.8	57.0	0.3	58.1
	60	3	3[f]	3.5	2.6	51.6	0.3	54.5
		6	6[g]	3.5	1.0	57.6	0	58.6
Cyclo-octane	25	15	3[e]	7	1.4	61.7	0.5	63.6
Toluene	25	10	2[e]	7	0.9	58.9	0	59.8

[a]The volume of 10% (w/v) M-040 was 2.00 ml, except where indicated otherwise; activity of CGTase was 0.6 unit/μl.

[b] 13% (w/v) of reaction mixture, except with cyclohexane at 50 °C and 60 °C, in which cases a concentration of 26% (v/v) was used.

[c] Maltodextrin M-050 was used for this run.

[d] Volume of 10% (w/v) M-040 was 4.00 ml; liquid volume of hydrocarbon was 3 ml; the hydrocarbon reacts with β-CD to form an insoluble complex that precipitates from solution during the course of the reaction.

[e] One per 5-day period.

[f] One per day.

[g] Two per day.

Table 3 Conversion of maltodextrin and corn-syrup solids in the presence of cyclic ketones[a]

Substrate	d.e.	D-Glucose content (%)	Maltose content (%)	Complexant	CD yields (%) 25 °C[b] α	β	γ	5 °C[c] α	β	γ
Maltodextrin										
M-040	5	0.5	0.5	None	8.3	16.0	2.7	4.6	7.9	2.1
				Cyclohexanone	1.7	58.0	0.2			
				Cyclo-octanone	0.4	66.0	0			
				Cyclodecanone[d]	0.4	62.7	2.8	0.2	63.0	6.0
				Cyclotridecanone	1.2	1.5	37.3			
				8-Cyclohexadecen-1-one	1.7	2.5	28.0			
Corn-syrup solids										
M-255	25	2.2	9.0	None	3.1	3.9	0.7			
M-250	25	8.3	7.3	None	2.4	3.2	0.5			
Fro-Dex 24	24	13.8	8.3	None	2.5	1.7	0.3			
Fro-Dex 42	42	25.1	14.7	None	1.1	1.5	0.1			
M-255	25	2.2	9.0	Cyclodecanone	0.2	42.8	0.2	0.2	42.1	<0.5
M-250	25	8.3	7.3	Cyclodecanone	<0.5	45.5	2.6	<0.5	43.4	0.6
Fro-Dex 24	24	13.8	8.3	Cyclodecanone	0.3	32.2	0.3	0.3	34.3	0
Fro-Dex 42	42	25.1	14.7	Cyclodecanone	0.4	18.0	0.4	<0.5	17.8	<0.5

[a] Volume of solutions, 2.00 ml; concentration of substrate, 10% (w/v); weight of complexant, 0.05 g, except for cyclotridecanone and 8-cyclohexadecen-1-one, which were 0.07 g and 0.09 g respectively.

[b] One 7 μl application of CGTase (equivalent to 4 units of activity) for mixtures without complexant; two 7 μl applications (at a frequency of one application per 5-day period) for two periods.

[c] One 15 μl application of CGTase (equivalent to 9 units of activity) for a period of 3 weeks.

[d] Yield of D-glucose at 25°C, 3.4%.

tion period, long after malto-oligosaccharides G_3–G_7 had made their appearance. Even then, cyclodextrin was present in barely trace amount as α-CD (0.3% yield), with the main reaction product being D-glucose (see Table 4 for the yields of D-glucose and unconverted maltose). At the much lower temperatures of 5 and 25 °C, and in the absence of complexant, conversion into CD was more easily achieved; however, combined yields were still low. At 60 °C, in the presence of the complexant cyclodecanone (specific for β-CD), the combined yield did not rise to a level higher than 6%. Only the combined use of a low temperature and a complexant (known to enhance β-CD production from maltodextrin) made possible the attainment of higher yields from maltose. As Table 4 shows, use of cyclononanone, cyclodecanone and cycloundecanone at 25 °C can provide β-CD yields as high as 34, 26, and 15% respectively. Certain other complexants that are normally very effective in enhancing β-CD yields from maltodextrin at both low and high temperatures were inexplicably ineffective. Among these normally useful complexants were cyclohexane, cyclo-octane, 2,2,4-trimethylpentane (iso-octane), n-heptane, and n-dodecane. Figure 1 illustrates the conversion of maltose into β-CD at 25 °C in the presence of cyclodecanone with a single, small application of CGTase. The time required under these conditions to reach a maximum yield of 26.4% was 36 days. That similar yields of 25.4 and 24.4% were obtained with much larger quantities of enzyme and proportionally shorter reaction times (Table 4) provided evidence for the high stability of *Bacillus macerans* CGTase under low-temperature reaction conditions.

Decan-1-ol, which enhances yields of α-CD from maltodextrin, was ineffective in enhancing yields of α-CD from maltose. Similarly, no enhancement of γ-CD yield occurred with cyclotridecanone and cyclododecanone, both of which

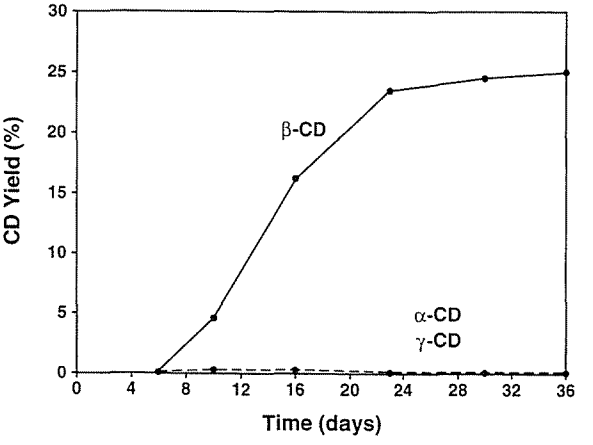

Figure 1

Conversion of maltose into α-, β-, and γ-CDs in the presence of cyclodecanone at 25 °C and pH 7.2 with single application of CGTase. Solution (2 ml) contains 10% (w/v) maltose, 0.05 g of cyclodecanone and 2 units of CGTase.

provide excellent yields (≈50%) of γ-CD from maltodextrin at 60 °C [9].

With malto-oligosaccharides larger than maltose, conversion into CD at low temperature occurs more readily than with maltose (see Table 4). Complexants that are known to be useful in enhancing yields of either α-CD or β-CD from maltodextrin may be employed. Yields of β-CD as high at 49% were achieved with maltotriose and as high as 66% with maltohexaose. With decan-1-ol as complexant to enhance α-CD yields, yields of α-CD from maltotriose and maltohexaose at 25 °C were 18 and 54% respectively.

Conclusion

Low-temperature conversion of maltodextrin into CDs in the presence of complexants that enhance α-CD and/or β-CD production provided yields that were at least comparable with, and often larger than, those obtained at much higher temperatures. All non-cyclic C_3–C_{15} hydrocarbons are highly specific for β-CD production, with

Table 4 Conversion of malto-oligosaccharides in the absence or presence of complexant[a]

Amounts of substrates were as follows: maltose, 0.585 mmol; maltotriose, 0.397 mmol; maltohexaose, 0.202 mmol. In rows 5 and 6 the amount of maltose was 0.602 mmol. Abbreviations: NOI, number of increments; IV, increment volume.

Substrate	Complexant	Temp. (°C)	Time (days)	CGTase		CD yield (%)				Glucose produced (mmol)	Maltose (mmol)
				NOI	IV (μl)	α	β	γ	Combined		
Maltose	None	60	2	1	3.5	0	0	0	0	0.003	0.570
	None		8	15	3.5	0	0	0	0	0.082	0.440
	None		26	50	3.5	0.3	0	0	0.3	0.428	0.200
	None		6	5	35.0	0	0	0	0	0.454	0.192
	None	25	6	1	3.5	0.5	0.1	0.1	0.7	0.120	0.435
	None		15	3	3.5	0.4	1.0	0.2	1.6	0.327	0.176
	None		13	1	10.5	0.5	0.5	0	1.0		
	None	5	7	1	15	0.7	0.5	0.2	1.4		
	None		14	1	15	1.2	0.5	0.1	1.8		
	None		21	1	15	1.2	0.4	0.1	1.7		
	Cyclodecanone	60	8	15	7	0.7	4.5	1.1	6.3		
		25	36	1	3.5	0	26.4	0	26.4	0.264	0.086[b]
			12	1	10.5	0	25.4	0	25.4		
			4	1	31.5	0.1	24.4	0	24.5		
	Cyclodecanone	5	7	1	15	0.3	2.5	0	2.8		
			14	1	15	0	15.4	0	15.4		
			21	1	15	0	30.2	0	30.2		
			28	1	15	0	30.5	0	30.5		
	Cyclononanone	25	50	10	3.5	0	34.2	0	34.2		
	Cycloundecanone	25	50	10	3.5	0.4	15.3	0.2	15.9		
	Cyclododecanone	25	50	10	3.5	1.5	2.1	0	3.6		
	Cyclotridecanone	25	50	10	3.5	1.8	1.6	0.1	3.5		
	n-Dodecane	25	10	10	3.5	1.8	1.3	0.1	3.2		
	Cyclohexane (0.5 ml)	25	20	1	10.5	0.1	2.1	0.1	2.3		
	n-Heptane (0.5 ml)	25	39	1	31.5	1.3	0.5	0	1.8		
		5	35	1	60	1.9	0.3	0	2.2		
	2,2,4-Trimethylpentane (0.5 ml)	25	25	5	7	0.2	0.3	0	0.5	0.336	0.164[c]
		5	28	1	15	0.2	0.2	0	0.4		
	Decan-1-ol (0.3 ml)	25	25	5	7	1.8	0.5	0	2.3		
		5	28	1	15	0.3	0.2	0.1	0.6		
Maltotriose	None	60	6	5	3.5	6.1	3.2	0	9.3		
	None	25	10	2	7	4.4	3.6	1.1	9.1	0.138	0.096
	Cyclodecanone	60	11	10	3.5	1.1	30.0	0.8	31.9		
		25	35	5	3.5	0	49.3	0	49.3		
	Cyclohexane (0.5 ml)	25	10	2	7	0.2	35.9	0	36.1		
	Cyclo-octane (0.5 ml)	25	35	5	3.5	1.4	28.5	0	29.9		
	2,2,4-Trimethylpentane (0.3 ml)	25	10	2	7	0	35.3	0	35.3	0.182	0.093
	Decan-1-ol (0.3 ml)	25	10	2	7	18.0	2.3	0	20.3	0.087	0.0485
Maltohexaose	2,2,4-Trimethylpentane (0.3 ml)	25	10	2	3.5	0.2	65.8	0	66.0	0.088	0.055
	Decan-1-ol (0.3 ml)	25	10	2	3.5	54.0	2.8	0	56.8	0.077	0.054

[a] Volume of solutions, 2.00 ml; concentration of substrate, 10% (w/v); amount of complexant, 0.32 mmol, except where indicated otherwise; where more than one increment of CGTase was applied, the frequency of application was no greater than two per day for reactions at 60 °C and one per 5-day period for reactions at 25 °C. The activity of CGTase was 0.6 unit/μl.

[b] Other malto-oligosaccharides present were maltotriose (0.025 mmol) and maltotetraose (0.008 mmol).

[c] Other malto-oligosaccharides observed were maltotriose (0.073 mmol), maltotetraose (0.034 mmol), maltopentaose (0.010 mmol) and maltohexaose (0.006 mmol).

branched hydrocarbons providing the highest yields (up to 64%). Alicyclic C_5–C_8 hydrocarbons also promoted the production of β-CD (65% yield). Excellent yields of β-CD (up to 59%) were possible with n-butane at low temperature, but not at the much higher temperature of 50 °C, where the yield was only moderate (31%). An advantage in using n-butane is the relative ease with which the encapsulated complexant is removed by gentle warming, thus eliminating the need for time-consuming azeotropic distillation.

All C_2–C_{14} alkyl alcohols greatly enhanced overall (combined α-, β- and γ-CD) yields at low temperature. Methanol enhanced the yield only slightly. Selectivity among the alcohols for a particular CD homologue (α-CD or β-CD) varied according to chain length and degree of branching. No alcohol enhanced γ-CD production. Production of α-CD at low temperature was highly favoured by unbranched alcohols having nine or more carbon atoms, with the greatest α-CD yields and selectivity being exhibited by C_9–C_{12} alcohols. Branched alcohols favoured high yields of β-CD.

A combination of low temperature and appropriate complexant permitted the conversion of low-molecular-mass malto-oligosaccharides into CDs in yields that varied from moderate to high. Maltose, maltotriose and maltohexaose were converted into β-CD in yields as high as 34, 49 and 66% respectively, which explained the observed beneficial influence of low temperature and complexant on the conversion of corn-syrup solids into β-CD. In the absence of low temperature, conversion of maltose into CDs is extremely poor.

Acknowledgments

Brand names are necessary to report factually on available data; however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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